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PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF ELECTION

(PCT Rule 61.2)

To:

Commissioner
US Department of Commerce
United States Patent and Trademark Office, PCT
2011 South Clark Place Room
CP2/5C24
Arlington, VA 22202
ETATS-UNIS D'AMERIQUE

in its capacity as elected Office

Date of mailing (day/month/year) 24 November 2000 (24.11.00)	
International application No. PCT/EP00/02718	Applicant's or agent's file reference 1723PTWO
International filing date (day/month/year) 28 March 2000 (28.03.00)	Priority date (day/month/year) 30 March 1999 (30.03.99)
Applicant SITAR, Giammaria	

1. The designated Office is hereby notified of its election made:

[X] in the demand filed with the International Preliminary Examining Authority on:

27 October 2000 (27.10.00)

[ ] in a notice effecting later election filed with the International Bureau on:

2. The election [X] was

[ ] was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO 34, chemin des Colombettes 1211 Geneva 20, Switzerland	Authorized officer C. Cupello
Facsimile No.: (41-22) 740.14.35	Telephone No.: (41-22) 338.83.38

Form PCT/IB/331 (July 1992)

EP0002718

09/937137

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
1723PTWO		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/EP00/02718	28/03/2000	30/03/1999
International Patent Classification (IPC) or national classification and IPC G01N33/49		
Applicant SITAR, Giammaria		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 5 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 4 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☐ Priority
   - III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations suporting such statement
   - VI ☐ Certain documents cited
   - VII ☒ Certain defects in the international application
   - VIII ☐ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
27/10/2000	18.06.2001
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized officer Klee, B Telephone No +49 89 2399 2675

Form PCT/IPEA/409 (cover sheet) (January 1994)

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/EP00/02718

I. Basis of the r port

1. With regard to the **elements** of the international application *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17))*:
Description, pages:

1,3-12	as originally filed			
2,2a	as received on	30/03/2001	with letter of	26/03/2001

Claims, No.:

1-6	as received on	30/03/2001	with letter of	26/03/2001

Drawings, sheets:

1/1	as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

   These elements were available or furnished to this Authority in the following language: , which is:

   - ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
   - ☐ the language of publication of the international application (under Rule 48.3(b)).
   - ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

   - ☐ contained in the international application in written form.
   - ☐ filed together with the international application in computer readable form.
   - ☐ furnished subsequently to this Authority in written form.
   - ☐ furnished subsequently to this Authority in computer readable form.
   - ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
   - ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

- ☐ the description, pages:
- ☐ the claims, Nos.:
- ☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

   (Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-6
	No:	Claims	
Inventive step (IS)	Yes:	Claims	1-6
	No:	Claims	
Industrial applicability (IA)	Yes:	Claims	1-6
	No:	Claims	

2. Citations and explanations
   see separate sheet

VII. Certain defects in the international application

The following defects in the form or contents of the international application have been noted:
see separate sheet

References cited

D1: US-A-5 663 051
D2: US-A-5 676 849
D3: US-A-5 489 386
D4: US-A-5 432 054

Re Item V

Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. With respect to claims 1 and dependent claims 2 to 6:
D3 describes a method for isolating fetal cells present in maternal peripheral blood (column 1, lines 42-48 and 50-55) comprising steps of
a) transferring maternal blood into non-physiological tissue culture medium (abstract, "hypertonic gradient medium")
c) isolation of the nucleated cells in a discontinuous density gradient (claim 1)
d) washing of the cells and resuspension in tissue culture medium (implicitly, column 9, line 55, 56)
e) identification of the cells by appropriate procedures and counting (column 10, line 22-24).
None of the documents cited describes the modification of the cell densities with a medium which after addition of an aqueus solution containing citric acid, Na citrate and dextran, has the following characteristics: pH6.4-6.6, osmolality 300-330mmol/l, Na+ 150-170mmol/l, K+ 4.5-5.5 mmol/l, Cl- 100-115 mmol/l, Ca++ 1.00-2.50 mmol/l, glucose 400-500 mg/dl, lactate 10-20 mg/d and the completion of the isolation of NRBCs with a single step procedure by combining steps a) addition of an aqueous solution containing citric acid, Na citrate and dextran and b) transfer into a cell separation device with a liquid having an higher density and containing RBCs aggregating agent.
Therefore claim1 and dependent claims 2 to 6 appear to be inventive.

Re Item VII

Certain defects in the international application

2. The amendments filed with the letter dated 26.03.01 introduce subject-matter which extends beyond the content of the application as filed, contrary to Article 34(2)(b) PCT. The amendments concerned are the following: Description page

2a, lines 12, 13. New statements of advantages of the invention are not permissible because they introduce into the description matter which could not have been deduced from the application as originally filed. Furthermore, the prior art should not be referred to in a manner likely to mislead.

polymerase chain reaction (PCR). Following these studies by molecular biology techniques, the presence of fetal cells in maternal blood has been later confirmed and is now firmly established. Embryonic cells (before XII weeks of gestation) and fetal cells (after XII weeks of gestation) are collectively termed "fetal cells" , in the international literature. Several procedures have been proposed in literature to isolate these few cells for non-invasive genetic investigation, but the final fetal cell yield is so low that it precludes reliable cytogenetic analysis by fluorescence in situ hybridization (FISH) or other genetic procedures due to an enormous maternal cell contamination.

The most favorable candidate cell type to be isolated for prenatal non-invasive genetic investigation is the nucleated red blood cell (NRBC), which is exceptionally rare in adult blood while in early fetal blood NRBCs are the most represented cell type together with stem cells.

Fetal white blood cells are present in extremely low percentage in fetal blood during the first trimester of pregnancy, it is therefore highly improbable to find them in maternal blood during the first trimester of pregnancy.

At least 20 fetal cells have to be isolated from maternal blood for reliable genetic investigation. According to the literature, few hundreds fetal cells are circulating in 25 ml maternal peripheral blood,

within 150-200.10^6maternal nucleated cells and 100-150. 10^9RBCs.

This exceedingly low number of fetal cells within a large bulk of maternal cells represents the major obstacle to be overcome especially in view of the fact that a multi-step procedure produces a cellular loss along each step. The preferred method must therefore try to minimize cellular loss and obtain fetal cell isolation by a single-step procedure.

Patents are known disclosing methods for enriching and isolating fetal cells from peripheral maternal blood.

USP 5,676,849 refers to a method for enriching a maternal whole blood sample for desired fetal cell population based on the density gradient centrifugation and passing the desired fraction containing fetal cell population through a counterflow stabilized charge-flow separator apparatus.

USP 5,489386 discloses a density gradient medium for the isolation of rare cells

including fetal nucleated erythrocytes from peripheral material blood, said medium comprising a colloidal density gradient medium dispersed in a meltable gel.

USP 5,432,054 refers to a method for isolating and enriching rare cells, including fetal nucleated erythrocytes from peripheral maternal blood, including two centrifugation steps.

The first centrifugation step has the aim of obtaining a red blood cell fraction.

The second centrifugation is carried out in a vessel containing a density gradient medium consisting of a colloid dispersed in a meltable gel.

After hemolysis of maternal red blood cells and melting the gel, the enriched fetal red blood cell fraction is centrifuged through a density gradient medium to obtain a fraction enriched in fetal red blood cells.

Said techniques are cumbersome, time-consuming, expensive and difficult to adapt to large scale screening or clinical testing applications. Moreover standard cell separation methods to isolate fetal cells from maternal blood use a first step whereby fetal cells are enriched by density gradient centrifugation in standard centrifuge tubes followed by highly sophisticated technology as centrifugal eluitration, fluorescence activated cell sorting or charge flow separation.

Density gradient centrifugation in standard centrifuge tubes produces major cellular loss, at least 50% of cells initially present in the starting cell sample hit the tube walls where they stick or aggregate falling down to the tube bottom),

CLAIMS

1. A method for isolating fetal cells present in maternal peripheral blood for prenatal genetic investigation, comprising the steps of:

a) transferring maternal blood into non-physiological tissue culture medium, which after addition of an aqueous solution containing citric acid, Na citrate and dextran, has the following characteristics:

pH	6.4 –6.6	
osmolality	300-330	mOsm
Na^+	150-170	mmol/l
K^+	4.5-5.5	mmol/l
Cl^-	100-115	mmol/l
Ca^{++}	1.00-2.50	mmol/l
glucose	400-500	mg/dl
lactate	10-20	mg/d

b) maternal blood, as modified in a) is transferred into a cell separation device, followed by the introduction into the said separation device of a liquid having an higher density and containing a RBCs aggregating agent;

c) the nucleated cells, having a lower density than the liquid introduced in the step b) are isolated, in the discontinuous density gradient, by subjecting the separation device to centrifugal force;

d) the isolated cells are washed and resuspended in tissue culture medium to regain physiological cell metabolism;

e) fetal cells are identified by appropriate procedures and counted.

2. The method of claim 1 whereby fetal NRBCs are isolated.

3. The method of claim 3 in which the non-physiological medium obtained in step a) has the following characteristics:

pH	6.5	
osmolality	320	mOsm
Na^+	165	mmol/l
K^+	5.35	mmol/l
Cl^-	110	mmol/l
Ca^{++}	1.25	mmol/l

glucose	500	mg/dl
lactate	10	mg/dl

4. The method of claim 1 in which the RBCs aggregating agent of step b) is Ficoll.

5. The method of claim 1 in which the density of the liquid introduced in the separation device by the step b) is 1.068 g/ml.

6. The method of claim 1 in which the separation device used in step b), comprises an elongated chamber (1), whose cross section decreases from the base towards the top, at least a first channel (2) one end of which opens into the said chamber near the said base and the other end is connected to a pressurized liquid source, and a second channel (3) one end of which opens into the same chamber (1) at the device top while the other end opens at the exterior of the device, the said device further comprising at least one additional channel (4), one end of which opens at a middle level of said chamber height and the other end opens at the exterior of the device .

PATENT COOPERATION TREATY

09/937137

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference	FOR FURTHER ACTION see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.	
1723PTWO		
International application No.	International filing date *(day/month/year)*	(Earliest) Priority Date *(day/month/year)*
PCT/EP 00/ 02718	28/03/2000	30/03/1999
Applicant		
SITAR, Giammaria		

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 3 sheets.

[X] It is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the report**

   a. With regard to the **language**, the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

   [ ] the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).

   b. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international search was carried out on the basis of the sequence listing :

   [ ] contained in the international application in written form.

   [ ] filed together with the international application in computer readable form.

   [ ] furnished subsequently to this Authority in written form.

   [ ] furnished subsequently to this Authority in computer readble form.

   [ ] the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

   [ ] the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

2. [ ] **Certain claims were found unsearchable** (See Box I).

3. [ ] **Unity of invention is lacking** (see Box II).

4. With regard to the **title**,

   [X] the text is approved as submitted by the applicant.

   [ ] the text has been established by this Authority to read as follows:

5. With regard to the **abstract**,

   [X] the text is approved as submitted by the applicant.

   [ ] the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority

6. The figure of the **drawings** to be published with the abstract is Figure No. 1

   [X] as suggested by the applicant.

   [ ] None of the figures.

   [ ] because the applicant failed to suggest a figure.

   [ ] because this figure better characterizes the invention.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP 00/02718

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 G01N33/49

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, WPI Data, BIOSIS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 5 663 051 A (VLASSELAER PETER VAN) 2 September 1997 (1997-09-02) abstract	1,2,5,8
A	column 1, line 10 - line 15 column 6, line 15 - line 25 column 6, line 50 - line 60 column 7, line 15 - line 27 column 9, line 45 - line 54 column 13, line 13 - line 45 column 21, line 62 -column 22, line 24 column 23, line 7 - line 29 claims 1,16	3,4,6,9, 10

-/--

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
18 July 2000	02/08/2000

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx 31 651 epo nl, Fax: (+31-70) 340-3016	Angelié, E

1

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
X	US 5 676 849 A (SAMMONS DAVID W ET AL) 14 October 1997 (1997-10-14) abstract column 1, line 10 - line 25 column 3, line 10 - line 22 column 4, line 15 - line 37 column 6, line 15 - line 30 column 6, line 51 - line 67 column 7, line 12 - line 18 column 8, line 27 - line 28 claim 1 ---	1,2,5,8
A	US 5 489 386 A (SAUNDERS ALEXANDER M) 6 February 1996 (1996-02-06) abstract column 1, line 51 -column 2, line 4 column 2, line 27 - line 29 column 2, line 65 -column 3, line 27 column 4, line 54 - line 65 column 6, line 44 - line 46 claim 1 ---	1-10
A	US 5 432 054 A (SAUNDERS ALEXANDER M ET AL) 11 July 1995 (1995-07-11) abstract column 1, line 47 -column 2, line 17 column 3, line 55 - line 64 column 5, line 51 - line 63 claim 1 -----	1-10

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/EP 00/02718

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 5663051 A	02-09-1997	US 5474687 A	12-12-1995
		US 5646004 A	08-07-1997
		US 5840502 A	24-11-1998
		US 5648223 A	15-07-1997
		AU 707878 B	22-07-1999
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		CA 2239729 A	19-06-1997
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		AT 186398 T	15-11-1999
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		EP 0739229 A	30-10-1996
		JP 3017291 B	06-03-2000
		JP 9508971 T	09-09-1997
		WO 9520429 A	03-08-1995
US 5432054 A	11-07-1995	AU 692838 B	18-06-1998
		AU 1689895 A	15-08-1995
		EP 0742836 A	20-11-1996
		JP 2977906 B	15-11-1999
		JP 9509312 T	22-09-1997
		WO 9520675 A	03-08-1995